

al., *Nature*, 345:739-743, 1990; Guilhot et al., *J. Bacteriol.* 176:535-539, 1994; Pelicic et al., *Proc. Natl. Acad. Sci. USA*, 94:10955-10960, 1997; Bardarov et al., *Proc. Natl. Acad. Sci. USA*, 94:10961-10966, 1997), among others. The specification also points to references explaining how to introduce mutations into the genome of bacteria in general (Ausubel et al., *Short Protocols in Molecular Biology*, 3<sup>rd</sup> ed., Wiley, 1995; Sambrook et al., *Molecular Cloning*, 2<sup>nd</sup> ed., Cold Spring Harbor Press, 1989), and texts discussing methods of mutagenesis of mycobacteria in particular (Jacobs et al., *Methods in Enzymology*, 204:537-555, 1991; Pelicic et al., *Molec. Microbiol.*, 28:413-420, 1998). As acknowledged by the Office, techniques for the mutation of bacteria are well known in the art. It would thus be routine for one skilled in the art to successfully introduce mutations into the genome of bacteria (or mycobacteria) using one of the techniques described in the specification.

The specification also provides guidance as to selecting successfully mutated bacteria. Since the focus of the present invention is on the identification of genes and nucleic acid sequences involved in virulence in bacteria, a selection method is used which selects those bacteria in which the mutation has disrupted a gene or nucleic acid sequence potentially involved in virulence. As the specification indicates, the selection may be based on the use of an antimicrobial agent, the effect of which is associated with a trait related to virulence, such as bacterial growth or DNA replication. Specific examples of this method are given in Examples 4 and 5, where *M. paratuberculosis* was cultured with the antimicrobial agent Bay y 3118 or D-cycloserine, which kill growing mycobacteria while having a reduced or no effect on non-growing bacteria.

The application of this selection technique can readily be applied to other species of mycobacteria and bacteria. Although the selection of an appropriate antibacterial agent may vary depending on the species of bacteria, methods for isolating bacterial mutants (particularly auxotrophic mutants) using antibiotics that kill growing cells is well known. See, e.g., Eisenstadt, et al., *Gene Mutation*, in *METHODS FOR GENERAL AND MOLECULAR BACTERIOLOGY* 297-316 (Gerhardt, et al., eds., 1997). Thus, selection of an appropriate antibacterial agent for the desired bacterial strain can be easily accomplished by those skilled in the art. In addition, as noted in the specification, the concentration of the antimicrobial agent used will vary with the particular substance and bacteria in question, but should exceed the approximate minimal inhibitory concentration (MIC). The determination of the MIC is routine in the art, and may be readily accomplished by a skilled technician. See, e.g., Lorian, *Antibiotics in Laboratory*

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*Medicine*, 4th ed., Williams and Wilkins, Baltimore (1996) (describing MIC and drug susceptibilities for many bacterial species).

The specification also provides examples of culture systems that can be used for mycobacteria, such as Middlebrook 7H9 broth, and intracellular culture systems such as a macrophage or amoeba culture system, as well as citations to references in which these methods are described (Middlebrook 7H9 broth--Jacobs et al., *Methods in Enzymology*, 204:537-55, 1991; Foley-Thomas et al., *Microbiology* 141:1173-81, 1995; and Williams, et al., *J. Clin. Microbiol.* 37:304-09, 1999; macrophage or amoeba culture system--Cirillo et al., *Infect. Immunol.* 65:3759-67, 1997). As the specification indicates, the exact amount of time required for culturing the bacteria with the antimicrobial agent will vary depending on the amount and type of selection agent used, the culture system, and the species of bacteria. However, such conditions can be optimized by a skilled technician through routine experimentation involving culturing bacteria for various times and at various concentrations of antimicrobials to determine the combination of times and concentrations which effectively kill growing but not non-growing bacteria. Non growing bacteria may then be readily selected. Useful selection methods are well known in the art. See, e.g., Rest and Speert, *Measurement of nonopsonic phagocytic killing by human and mouse phagocytes*, in BACTERIAL PATHOGENESIS 475-92 (Clark and Bavoli, eds., 1997) (describing methods for intracellular killing assays for bacteria within phagocytic cells); Bermudez, et al., *FEMS Microbiol. Lett.* 178(1):19-26, 1999 (describing a strategy to isolate mutants that grew in macrophages, using an antibiotic, for *M. avium*).

In addition, the specification provides guidance on testing the selected surviving bacteria for virulence. Specific examples of virulence testing are given in Examples 6 and 7, where the virulence of mutated and non mutated *M. paratuberculosis* is tested in beige mice. However, the techniques used for testing the virulence of *M. paratuberculosis* can readily be applied to other species of mycobacteria and bacteria. The specification teaches that virulence may be tested by inoculating the organism's natural host or an animal model with the selected organism and determining if the inoculation results in clinical symptoms or if the organism multiplies and spreads beyond the site of inoculation. Although the amount of the organism inoculated will vary with factors such as the species, size, and age of the animal, the appropriate amount of the organism to be administered can be determined by one of ordinary skill in the art through routine experimentation. In addition, routes and methods of administering compositions such as those claimed are well known. The specification provides examples of these routes and methods of

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administration, as well as citations to reference texts for the formulation of drugs (Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania (1975); Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y. (1980)).

The specification of the present invention has thus not only provided a specific example of a non-virulent strain of bacteria produced by the claimed process (*M. paratuberculosis*), but also provides sufficient guidance to enable one skilled in the art to apply this process to other strains of mycobacteria and bacteria. As discussed above, methods for introducing mutations into the genome of mycobacteria and bacteria, isolating mutants based on drug selection, virulence testing, and drug formulation are all well known in the art, and may readily be accomplished with reference to standard texts describing such methods, and routine experimentation. In addition, routine experimentation, such as that described above, is not considered "undue." MPEP §2164.06 states, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *See In re Wands*, 8 U.S.P.Q.2D 1400, 1406 (1988)(necessity of screening hybridomas to practice claimed invention not undue experimentation since the nature of the technology involved such screening, there was a high level of skill in the art, methods needed to practice the invention were well known, and the disclosure provided considerable direction and guidance); *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 U.S.P.Q.2d 1217, 1222-23 (Fed. Cir. 1988) (specification setting forth one embodiment plus the general manner in which its range was ascertained is enabling).

The specification thus provides sufficient guidance to enable others to create mutated bacteria, as well as the immunizing compositions of the present invention. Absence of a working example alone is not sufficient justification to find the specification non-enabling. MPEP §2164.02 states, "Compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed." So long as "the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation," *id.*, it is not necessary for the specification to contain a working example of the invention claimed.

As the court in *In re Wright*, 999 F.2d 1557, 1562, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993), notes, "the PTO bears an initial burden of setting forth a reasonable explanation as to why

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it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application.” In the present case, the examiner has not provided such an explanation.

The Office's assertion that “there is a lack of predictability in the art for the particularly claimed invention” is not supported by the record. The examiner himself states that “[t]he art of mutation of bacteria is well known, and the art of mutation of *Mycobacteria* in particular has been increasing in the near past.” But no reason or support is given to show why, given the state and skill level of the art, there is a lack of predictability for the particularly claimed invention; the existence of such a lack of predictability is simply asserted.

**VERSION WITH MARKINGS SHOWING CHANGES MADE**

**IN THE SPECIFICATION:**

Table 1 on page 25.

TABLE 1

**Mutation of *M. paratuberculosis* through Transposon Mutagenesis**

Expt. <sup>1</sup>	OD <sub>600</sub> culture <sup>2</sup>	Ratio[n] Ph/B <sup>3</sup>	Ads Time (hours) <sub>4</sub>	Transd. Freq. <sup>5</sup>	Transp Freq <sup>6</sup>	Kan <sup>r</sup> mutants <sup>7</sup>	N <sup>8</sup>	P value <sup>9</sup>
I	0.48	20	4	5.4x10 <sup>-8</sup>	1.0x10 <sup>-6</sup>	1344	1344	26%
II	0.48	200	4	5.1x10 <sup>-9</sup>	9.8x10 <sup>-7</sup>	1275	2619	42%
III	0.38	25	6	7.0x10 <sup>-9</sup>	1.8x10 <sup>-7</sup>	176	2795	46%
IV	0.38	25	24	5.4x10 <sup>-9</sup>	1.4x10 <sup>-7</sup>	136	2931	48%
V	0.75	350	4	1.1x10 <sup>-9</sup>	3.6x10 <sup>-5</sup>	2689	5620	71%

Footnote 8 on page 25.

<sup>8</sup> [Cumulative] **Cumulative** number of [kanamycin] **kanamycin**-resistant colonies obtained

Table 2 on page 26.

TABLE 2

**Virulence Testing of *M. paratuberculosis* in Susceptible Mice**

Week	CFU/g (mean of all mice in group $\pm$ SEM)					
	Liver		Spleen		Ileum	
	I	II	I	II	I	II
1	$5.9 \pm 0.6 \times 10^7$	$7.9 \pm 0.4 \times 10^1$	$2.7 \pm 0.3 \times 10^7$	$1.6 \pm 0.3 \times 10^2$	$3.5 \pm 0.6 \times 10^4$	$5.9 \pm 0.6 \times 10^3$
2	$1.4 \pm 0.8 \times 10^8$	$1.5 \pm 0.5 \times 10^1$	$1.1 \pm 0.4 \times 10^8$	$1.8 \pm 0.4 \times 10^2$	$4.0 \pm 0.3 \times 10^4$	$2.2 \pm 0.2 \times 10^4$
3	$2.5 \pm 0.4 \times 10^8$	Not done	$1.7 \pm 0.5 \times 10^8$	Not done	$9.9 \pm 0.6 \times 10^4$	Not done
4	Not done	$3.0 \pm 0.2 \times 10^2$	Not done	$1.6 \pm 0.3 \times 10^2$	Not done	$2.5 \pm 0.2 \times 10^4$
8	$2.9 \pm 0.6 \times 10^8$	$1.1 \pm 0.6 \times 10^3$	$5.6 \pm 0.4 \times 10^8$	$5.9 \pm 0.4 \times 10^3$	$2.2 \pm 0.3 \times 10^5$	$1.3 \pm 0.3 \times 10^4$

Fifth paragraph on page 5.

Figure 2 shows the location of the BETH-R and BETH-F primers in the Tn5367 transposon and partial results of sequencing. Also shown is the alignment of nucleotide sequence obtained from mutant GPM207 (SEQ ID NO: 5) using BETH-F and the *xerC* gene (GenBank No. Z97369) (SEQ ID NO: 6), **as well as the sequences at the transposon-chromosomal junction (SEQ ID NOS: 3 & 4).**

Sixth paragraph on page.

Figure 3 shows the effects of co-culture with either Bay y 3118 or D-cycloserine on growing (**Bay y 3118, Fig. 3B; D-cycloserine, Fig. 3D**) and non-growing (**Bay y 3118, Fig. 3A; D-cycloserine, Fig. 3C**) *M. paratuberculosis* strain K-10 in complete Middlebrook 7H9 medium. **Concentrations of Bay y 3118, D-cycloserine, and the control (0X) are as given in Example 4.**

**IN THE CLAIMS:**

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23. (once amended) The composition of claim 22, wherein said Bay y 3118 is used at a concentration **[between]** of at least 0.015  $\mu\text{g/mL}$ .

CONCLUSION

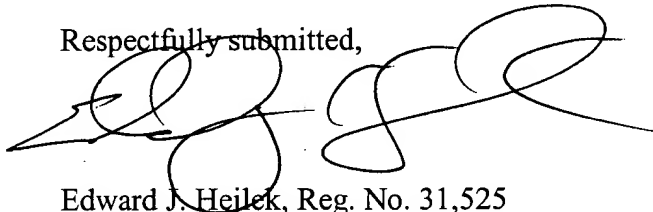
In light of the foregoing considerations, applicant respectfully request reconsideration of the rejection of claims 15-42 on the basis of 35 U.S.C. §112, second paragraph.

Pursuant to 37 C.F.R. 1.17 and 1.136(a), Applicants respectfully request a two month extension of time for filing a response to the above-mentioned Office action.

\* A check in payment of the extension fee is enclosed.

The Commissioner is hereby authorized to charge any underpayment and credit any overpayment of government fees to Deposit Account No. 19-1345.

Respectfully submitted,



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\*Enclosures

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